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HCV
Written in our DNA

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An inspection of the sequence similarity between the hepatitis C virus (HCV) polyprotein and human proteins revealed a high level of peptide sharing, with a limited number of motifs unique to the virus (i.e., with no counterpart in the human proteome). Using pentapeptide matching, only 214 motifs out of a total of 3,007 (7.11%) identified HCV as nonself compared to the *Homo sapiens* proteome. However, this virus-versus-human phenetic difference disappeared at the genetic level. Indeed, a BLAST analysis of pentadecameric oligodeoxynucleotide sequences corresponding to the 214 pentapeptides unique to HCV revealed that almost all of them are present in the human genome, located in the non-coding strand, introns, and/or pseudogenes, thus being, as such, untranslatable. The present data warn against using DNA-based vaccines to fight HCV infection and emphasize peptide uniqueness as the molecular basis for designing effective anti-HCV immunotherapeutic approaches.

The immune system acts to protect against infectious agents while preserving the integrity of host function.[1,2] Theoretical immunology states that, to properly function, the immune system must be able to discriminate what has to be defended (the so-called "self") from what has to be attacked and destroyed (i.e., the others, the enemies, the pathogens; in a word, the "nonself").[3] Hence, immunology is the classification of self and nonself entities.[4] According to the conceptual view of an immune system that has evolved to discriminate infectious nonself from noninfectious self,[5] viruses represent nonself entities par excellence. Viruses are small biological entities that must infect a suitable host cell in order to replicate. In other words, viruses are simple models (i.e., small molecules where replication is easily switched on/off in the host cell under suitable conditions) used to study the self/nonself immunological dichotomy. Nonetheless, defining the pathogen-host relationship(s) between viruses and animals is still the most arduous challenge in immunology and biomedicine. Chronic viral infections remain a serious health problem.[6] The scenario is alarming, first, because the spread of infectious agents is increasing and new viral pathologies are (re)emerging,[7-9] and second, because only an exact definition and clear understanding of virus-human relationships can provide the scientific knowledge necessary to build effective and safe immuno-biological therapeutic approaches.[10,11]

Given these premises, Kanduc and others[12-20] have searched viral proteomes for sequence (dis)similarities with the human proteome, the ultimate goal being to gain data on a peptide platform as the basis of the self-nonself delimitation between viruses and humans. Using a sample formed by 30 viral proteomes and utilizing the pentapeptide as a unit of length, it has been shown that: (1) Pentapeptides from viral antigens are massively disseminated among human proteins. On the whole, the viral versus human proteome overlap at the 5-mer level amounts to 2,907,096 matches (including multiple occurrences), and involves almost all the human proteins. (2) For each virus, only a limited number of viral pentapeptides have no counterpart in humans; therefore, they represent molecular signatures of the virus.[12]

From a clinical point of view, these data are important since using peptide motifs unique to viral proteins may represent an elective methodology for developing targeted, effective and safe vaccines against viral diseases.[10,11] Indeed, current anti-viral immunotherapeutic approaches might carry undesired side effects, possibly affecting essential cellular functions since most human proteins involved in the viral overlap are associated with basic cell functions such as proliferation, development and differentiation.[13-20] Scientifically, the existence of pentapeptide sets unique to viruses offers a methodological approach for defining the self-nonself boundaries that separate viruses and humans, and possibly, understanding the basic principles underlying viral infections and pathogenesis.

Proceeding in this research direction, the present study describes a pentapeptide platform that specifically marks the HCV polyprotein, and moreover, moves research a step forward by demonstrating that the oligodeoxynucleotide sequences corresponding to HCV-specific pentapeptide motifs (i.e., not expressed in the human proteome) are actually part of the human genome.

Results

HCV-versus-human self-nonself at the phenetic level. Our similarity analyses utilized pentapeptides as probes since a 5 amino acid grouping is the minimal antigenic space sufficient to

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specify an immune reaction; that is, 5 amino acids can delimit an immune unit.[21-30] This definition delineates "self" as the pentapeptide repertoire present in an organism, and vice versa, "nonself" as the pentapeptide set absent in the organism.[31] Namely, in an immunological context, the pentapeptides unique to HCV and absent in human proteins demarcate the self/nonself boundaries between the virus and *Homo sapiens*.

Following PIR sequence similarity analyses[32] of the HCV1a polyprotein[33] and human proteome as described in the Methods, we determined the HCV self as a set of 214 pentapeptides. In essence, only 214 motifs out of 3,007 viral pentapeptides specify the HCV proteome, while the remaining 2,793 are widely and repeatedly diffused throughout the human proteome.[12,13] **Table 1** presents the pentapeptides unique to HCV1a.

HCV-versus-human self-nonself at the genetic level. We also analysed HCV-versus-human uniqueness at the genetic level. DNA is the ultimate repository of information that specifies an organism. Basically, DNA is a cumulative sequence structure that specifically barcodes each organism and its evolution. We reasoned that, if a peptide distinction exists between two entities, it must be certified at the nucleic acid level. Otherwise stated, if the 214 pentapeptides unique to HCV are the mark distinguishing the virus from its human host, then such a difference must become evident in a comparative analysis of their DNA.

Therefore, using the standard nucleotide-nucleotide BLAST (blastn) program[34] as described in the Methods, a nucleotide sequence similarity search was conducted for occurrences of the oligodeoxynucleotide sequences encoding HCV1a pentapeptides not expressed in the human proteome (**Table 1**). **Table 2** is an *in extenso* description of the results obtained for the pentapeptide RTWAQ unique to HCV1a and absent in the human proteome (see the first pentapeptide in **Table 1**). The oligodeoxynucleotide sequence corresponding to the pentapeptide RTWAQ (5'-AGGACCTGGGCTCAG-3') was repeatedly present in human DNA, disseminated among different chromosomes. Specifically, the sequence 5'-AGGACCTGGGCTCAG-3' was located in introns, frameshifts and the DNA minus strand (i.e., in untranslated regions or non-coding DNA) for a total of 45 occurrences.

These results applied to almost all of the 214 unique HCV1a pentapeptides. **Supplemental Table 3** illustrates that practically all of the pentadecameric oligodeoxynucleotide sequences corresponding to unique HCV1a pentapeptides occur repeatedly in untranslated, frameshifted, and/or non-coding human DNA. The only exceptions were: 42 oligodeoxynucleotides represented at the tetradecameric level, eight oligodeoxynucleotides at the tridecameric level and one oligodeoxynucleotide (i.e., 5'-ATGAGGATCGTCGGT-3' corresponding to the HCV1a pentapeptide MRIVG [aa 2,043–2,047]) present 89 times at the dodecameric level (**Sup. Table 3**). In essence, by examining the HCV1a pentapeptide set at the nucleotide level, we found that the maximum genetic difference between HCV and humans is, at most, represented by trinucleotides.

It should be noted that in the case of consecutive overlapping pentapeptides, the corresponding oligodeoxynucleotide sequence in the human genome exceeded 15 nucleotides in length (see, for example, in **Table 1** the viral decapeptide AWDMMMNWSP [aa318–327] consisting of 6 consecutively overlapping pentamers with zero similarity to the human proteome).

Discussion

During the last decade we have learnt much about HCV and its interactions with its human host. We know that entry of HCV into host cells involves cellular factors;[35] HCV core protein affects host antiviral and immune responses, and consequently, modulates HCV pathogenesis;[36] HCV induces the unfolded protein response, which in turn activates the autophagic pathway to promote HCV RNA replication in human hepatoma cells;[37] a peptide derived from the N terminus of HCV1a NS5A (SWLRDIWDWICEVLSDFK) inhibits HCV infection in vitro;[38] and so on. These data indicate that an understanding of the relationships between HCV and its human host at the molecular level is fundamental to preventing and/or controlling the HCV infectious disease.

Based on these premises, the present study involved a mathematical dissection of the (un)commonalities between HCV and *Homo sapiens* to provide an operative platform for designing therapeutic approaches based on specific sequences and functions. The results presented here expand upon peptide-to-peptide profiling of the HCV polyprotein versus the human proteome and clearly define the peptide boundaries of HCV self as a set of 214 pentapeptides unique to the virus. In addition, data are reported showing that the viral-vrs-human distinction is not present at the genetic level. Indeed, the lack of occurrence of the 214 unique HCV1a pentapeptides in the human proteome is not due to a lack of the corresponding pentadecameric oligodeoxynucleotides in human DNA. Noticeably, almost all of the oligodeoxynucleotides corresponding to the unique HCV1a pentapeptides are present in human DNA, although they're not expressed due to their location in untranslated DNA regions or in the minus noncoding strand. That is, the barcodes distinguishing HCV and humans do not appear to reside in two unique DNA sequence collections. Rather, it seems that the self-nonself issue is determined by a physical space dimension; the oligodeoxynucleotides are located in non-coding or untranslated regions of human DNA leading to no expression in the human proteome of the unique HCV1a pentapeptides.

Hence, a first consideration emerging from this study is evolutionary in nature. Our results suggest that HCV and humans derived their genetic and functional information from a common ancestral template; thus, this study can be framed in the viral eukaryogenesis hypothesis, according to which the primordial eukaryotic cell might be represented as a multimember consortium consisting of a viral ancestor of the nucleus, an archaeal ancestor of the eukaryotic cytoplasm and a bacterial ancestor of mitochondria.[39,40]

Second, the viral-versus-human oligodeoxynucleotide overlap might interfere with nucleic acid amplification testing and contribute to false-positive results in HCV medical diagnoses and blood screening. Also, the present data are of special importance when considering the current new DNA

Table 1. Peptide analysis of the HCV1a primary sequence versus the human proteome: unique HCV identity spots at the pentapeptide level

Pos[1]	5-mer[2]	Pos[1]	5-mer[2]	Pos[1]	5-mer[2]	Pos[1]	5-mer[2]	Pos[1]	5-mer[2]
73	RTWAQ	548	WFGCT	855	QLHVW	1603	WDQMW	2099	HYVTG
74	TWAQP	549	FGCTW	856	LHVWI	1604	DQMWK	2110	KCPCQ
79	GYPWP	551	CTWMN	857	HVWIP	1605	QMWKC	2113	CQVPS
92	WAGWL	552	TWMNS	858	VWIPP	1607	WKCLI	2151	HEYPV
131	DLMGY	573	AGNNT	876	MCAVH	1641	TKYIM	2236	MGGNI
200	YHVTN	579	HCPTD	877	CAVHP	1643	YIMTC	2286	VWARP
202	VTNDC	581	PTDCF	899	WILQA	1644	IMTCM	2287	WARPD
229	VREGN	601	WITPR	931	HYVQM	1645	MTCMS	2310	VVHGC
237	CWVAM	606	CLVDY	946	TYVYN	1670	AAYCL	2415	DVVCC
258	RRHID	609	DYPYR	956	RDWAH	1718	EQGMM	2418	CCSMS
295	RRHWT	610	YPYRL	982	LITWG	1719	QGMML	2423	YSWTG
297	HWTTQ	611	PYRLW	983	ITWGA	1749	VQTNW	2431	TPCAA
298	WTTQG	612	YRLWH	985	WGADT	1750	QTNWQ	2451	RHHNL
315	HRMAW	613	RLWHY	1013	PADGM	1760	WAKHM	2452	HHNLV
316	RMAWD	614	LWHYP	1074	NGVCW	1761	AKHMW	2463	SACQR
318	AWDMM	615	WHYPC	1075	GVCWT	1762	KHMWN	2465	CQRQK
319	WDMMM	618	PCTIN	1077	CWTVY	1763	HMWNF	2538	INSVW
320	DMMMN	627	KIRMY	1079	TVYHG	1764	MWNFI	2557	IMAKN
321	MMMNW	628	IRMYV	1098	QMYTN	1814	WVAAQ	2562	EVFCV
322	MMNWS	630	MYVGG	1106	DLVGW	1911	AVQWM	2634	MGFSY
323	MNWSP	641	AACNW	1130	YLVTR	1914	WMNRL	2638	YDTRC
363	MVGNW	642	ACNWT	1184	CTRGV	1958	RLHQW	2639	DTRCF
364	VGNWA	643	CNWTR	1201	ETTMR	1960	HQWIS	2641	RCFDS
425	ALNCN	644	NWTRG	1266	YMSKA	1978	DIWDW	2659	YQCCD
427	NCNDS	645	WTRGE	1270	AHGID	1979	IWDWI	2695	YRRCR
432	LNTGW	668	TTQWQ	1291	TYSTY	1980	WDWIC	2713	TCYIK
440	LFYHH	670	QWQVL	1316	ECHST	1982	WICEV	2728	QDCTM
441	FYHHK	711	WAIKW	1387	GRHLI	2017	VWRVD	2731	TMLVC
443	HHKFN	713	IKWEY	1391	IFCHS	2022	GIMHT	2761	AMTRY
464	FDQGW	727	DARVC	1436	TDALM	2024	MHTRC	2762	MTRYS
482	RPYCW	732	SCLWM	1448	DSVID	2025	HTRCH	2785	CSSNV
483	PYCWH	733	CLWMM	1449	SVIDC	2026	TRCHC	2801	YYLTR
484	YCWHY	770	FFCFA	1451	IDCNT	2029	HCGAE	2827	WLGNI
485	CWHYP	772	CFAWY	1455	TCVTQ	2043	MRIVG	2832	IMFAP
486	WHYPP	786	VYTFY	1520	YDAGC	2050	TCRNM	2844	LMTHF
488	YPPKP	787	YTFYG	1522	AGCAW	2051	CRNMW	2845	MTHFF
492	PCGIV	789	FYGMW	1523	GCAWY	2052	RNMWS	2863	DCEIY
504	PVYCF	790	YGMWP	1524	CAWYE	2053	NMWSG	2864	CEIYG
505	VYCFT	791	GMWPL	1549	VCQDH	2058	TFPIN	2898	PGEIN
506	YCFTP	803	PQRAY	1553	HLEFW	2059	FPINA	2917	RAWRH
524	PTYSW	837	YISWC	1556	FWEGV	2074	APNYT	2967	SGWFT
525	TYSWG	840	WCLWW	1566	HIDAH	2075	PNYTF	2990	WIWFC
547	NWFGC	843	WWLQY	1567	IDAHF	2079	FALWR		

HCV1a pentapeptides with zero similarity to the human proteome are sequentially listed by amino acid position along the HCV1a primary sequence. [1]Amino acid position along the HCV1a polyprotein. [2]Amino acid sequence in one-letter code of the zero-similarity pentapeptides.

Table 2. Location in the human genome of the pentadecameric oligodeoxynucleotide 5'-AGGACCTGGGCTCAG-3' sequence corresponding to the pentapeptide RTWAQ unique to HCV1a and absent in the human proteome

DNA strand	NCBI ref	Chromosome	Gene	Nucleotide position
Plus	NT_167186.1	1	ARV1 (intron)	24643405–24643419
Plus	NT_032977.9	1		3153192–3153206
Plus	NT_032977.9	1	KCNQ4 (intron)	11239419–11239433
Minus	NT_021937.19	1		1034121–1034107
Plus	NW_001838549.1	1		653918–653932
Minus	NW_001838577.2	1		1882633–1882619
Minus	NW_001838577.2	1		9971849–9971835
Plus	NW_001838640.2	1		191744–191758
Plus	NT_005403.17	2	DIS3L2 (intron)	83412729–83412743
Plus	NT_005403.17	2		83523475–83523489
Minus	NT_022184.15	2		15060499–15060485
Minus	NW_001838867.2	2		689522–689508
Minus	NW_001838867.2	2		800295–800281
Minus	NW_001838769.1	2		6068391–6068377
Plus	NT_016354.19	4		35524624–35524638
Plus	NW_001838915.1	4		35470558–35470572
Minus	NT_006576.16	5		40667409–40667395
Minus	NW_001838933.1	5		4972008–4971994
Minus	NT_007914.15	7		10013400–10013386
Minus	NW_001839078.1	7		5374522–5374508
Plus	NT_008046.16	8		57548408–57548422
Minus	NT_008183.19	8		1108835–1108821
Minus	NW_001839142.2	8		1890517–1890503
Minus	NW_001839132.1	8		1107463–1107449
Minus	NM_182487.2	9		1146–1132
Minus	NT_019501.13	9		271360–271346
Minus	NT_008470.19	9		56731018–56731004
Minus	NW_001839244.1	9		74917–74903
Minus	NW_001839239.1	9		2906164–2906150
Minus	NT_030059.13	10		31696610–31696596
Plus	NW_001837987.2	10		568360–568374
Minus	NT_009237.18	11		45886294–45886280
Plus	NW_001838022.2	11		4963111–4963125
Minus	NT_026437.12	14		70923235–70923221
Plus	NT_026437.12	14	EVL (intron)	81551311–81551325
Minus	NW_001838115.2	14		4148419–4148405
Plus	NW_001838113.2	14		5812026–5812040
Minus	NT_010194.17	15		13103553–13103539
Minus	NT_010194.17	15		35134868–35134854
Plus	NW_001838218.2	15		10022041–10022055
Plus	NW_001838214.2	15	PLA2G4E (intron)	1558027–1558041
Minus	NT_010783.15	17		28728099–28728085
Plus	NW_001838454.2	17		12060283–12060297
Minus	NT_011387.8	20		8436891–8436877
Minus	NW_001838652.1	20		8448265–8448251

immunisation approaches for HCV. Based on the principle of incorporating genes from a pathogen into the genome of a normally nonpathogenic organism for amplification of the immunogens, DNA vaccines have been proposed for viruses like HCV that cannot be grown in cell culture.[41,42] In light of the present data, a possible outcome of such DNA vaccine approaches might consist of evoking antibodies against human DNA sequences.

In conclusion, this study reinforces the concept that only a vaccine formulation based on peptide sequences uniquely present in the virus and absent in the proteins of the host have the potential to hit HCV and halt the viral infection, without causing collateral harmful cross-reactions. Hence, by having zero similarity to the human proteome, the unique HCV peptides described in **Table 1** constitute an ideal basic peptidome platform that could result in the long-sought anti-HCV vaccine. In this regard, it is also worth of note to recall that a comprehensive meta-analysis of data from the literature on HCV epitope mapping has revealed that the B cell epitope repertoire is allocated to rare peptide motifs, i.e., antigenic peptide sequences endowed with a low level of similarity to the host proteome.[27]

Indeed, two central desiderata mark an ideal immunotherapeutic approach: specificity and absence of collateral harmful effects. Lack of these requisites may underlie the current difficulties in developing effective anti-HCV vaccines, in face of the numerous approaches.[41-46]

Methods

The HCV nucleotide and amino acid sequences analysed in this study correspond to HCV, subtype 1a, taxon: 31,646, deposited in the GenBank database under accession no. M62321.[33] The polyprotein, which has a total length of 3,011 amino acids, consists of the following proteins: capsid C; envelope glycoprotein E1; envelope glycoprotein E2; p7; protease NS2-3; serine protease/NTPase/helicase NS3; non-structural (NS) 4A; NS4B; NS5A; and RNA-directed RNA polymerase.

Sequence similarity analyses comparing the viral polyprotein sequence to the human proteome were conducted using HCV pentapeptides as probes to scan the *Homo sapiens* proteome for exact matches. The scans were performed using the Protein Information Resource perfect match program (pir.georgetown.edu/pirwww/search/peptide.shtml).[32] HCV pentapeptide probes were offset by one amino acid residue; that is, overlapped by 4 residues so that 3,007 consecutive 5-mer peptide blocks (e.g., MSTNP, STNPK, TNPKP and NPKPQ) were analysed. For each viral 5-mer, the entire human proteome was searched for instances of this 5-mer.

Viral pentapeptides absent in the human proteome were singled out for further investigation at the genomic level using the Basic Local Alignment Search Tool (BLAST) program. Specifically, for each pentapeptide unique to HCV, the corresponding oligodeoxynucleotide sequence derived from HCV sequence, GenBank database, accession no. M62321,[33] was used to search the entire human genome for instances of the same nucleotide sequence. Oligodeoxynucleotide scans were carried out using the BLAST (blastn) program from the NCBI (http://blast.ncbi.nlm.nih.gov) to find and localize regions of 100% similarity (i.e., with no gaps allowed) in the human nucleotide collection covering genomic and transcript sequences.[34]

Note

Supplemental materials can be found at:
www.landesbioscience.com/journals/selfnonself/article/15795

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